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厦门大学

硕　士　学　位　论　文

富含 β-胡萝卜素的杜氏盐藻的技术开发研究

The study on the technology and development of *Dunaliella* which are riched in β-carotene

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摘 要

微藻在能源、医药、生物技术等有很大的应用前景，为了实现微藻的大规模工业化生产，微藻的培养、采收工艺、提取技术成为当前的研究重点。与其他藻种相比，盐藻能有效的积累天然 β-胡萝卜素，其产品开发呈现多样化趋势，具有较高的经济价值，因而受到广大研究者的重视。本研究对盐藻室内生长条件及 β-胡萝卜素积累条件进行考察;对室外条件下盐藻生长以及 β-胡萝卜素积累情况进行监测；构建了一套规模化养殖工艺；提出了两种盐藻分离工艺；建立了植物油微波提取 β-胡萝卜素的工艺。研究得到以下主要结论：

（1）室内条件下（6000Lux，30℃），以硝酸钠、硝酸钾作氮源，氯化钠调节盐度培养 7-8 天，盐藻干重可以达到 0.13 g/d,盐藻细胞生长的最佳条件为：氮浓度为 5.0mM、盐度为 1.5M、橘黄色光质。室内条件下（22000Lux，35℃），β-胡萝卜素积累的最佳条件为 0.5mM 的氮浓度、盐度 2.5M，β-胡萝卜素含量可以达到 3.64%，经过 8 天的培养β-胡萝卜素产量为 3.035mg/（L·d）。

（2）室外培养时（从 2015.05.29-2015.06.15，参数见附录表 3），以硝酸钠、硝酸钾作氮源，氯化钠调节盐度培养 18 天左右，β-胡萝卜素积累的最佳条件为 0.6mM 氮源，2.5M 盐度，积累的β-胡萝卜素含量为 3.14%,产量为 2.47 mg/（L·d）。

（3）二步法培养下藻细胞的β-胡萝卜素明显高于常规方法培养的藻细胞，比常规培养的β-胡萝卜素高出 33.2%；常规培养的藻细胞增长速度较快，最高达到 2.1×10^{7}cells/mL，比二步法培养下的藻细胞数量高出约 21.05%，这说明了极端环境会抑制盐藻细胞的生长。

（4）采用沙载电絮凝工艺和螺旋藻废液可以有效低耗的采收盐藻，当通电电流为 0.5A，沙子剂量 250mg/L，转速 150 rpm 时的可以得到最佳采收率 97.16%，能耗为 0.380 kWh/kg，远低于工业中应用的离心分离的 1.67 kWh/kg，采收过后的上清液经过氮浓度补充可以循环利用，能够节约水资源，降低培养成本；采用螺旋藻废液采收工艺时，在螺旋藻上清液与盐藻液比值为 1:4 时，采收效果最高可达到 75.26%，在螺旋藻上清液和培养基的混合液与盐藻的配比为 1:5 时，沉降时间为 3h 时，混合液的最佳采收率为 74.77%，将螺旋藻上清液絮凝盐藻液后的上层澄清液进行培养盐藻细胞时，配比为 2:5 的藻细胞生物量高出正常培养的

75%。

（5）植物油萃取工艺是一种绿色安全有效的β-胡萝卜素萃取工艺。植物油直接萃取工艺在反应条件为温度 50℃、油浴时间 40min、藻油比 1:5，最大采收率为 67.82%；植物油微波辅助萃取工艺在反应条件在功率 50%、微波加热时间 6min、温度 48℃、藻油比（微藻与植物油比例）1:6 时的最高萃取率可以达到 83.60%，比直接油浴法高出 23.27%；

关键词：盐藻；规模化培养；采收；β-胡萝卜素

Abstract

Microalgae have promising applications in the energy industry, pharmaceutical industry, and biotechnology. In order to achieve large-scale industrial production salina, microalgae cultivation, harvesting technology, extraction technology has become the focus of current research. Compared with other species of algae, *Dunaliella* which possesses a high economic value can effectively accumulate natural β- carotene, and its products showing a diversified trend. In this study, firstly we studied the growing conditions and β- carotene accumulation condition of *Dunaliella* indoor. Then, we studied corresponding conditions of growth and β- carotene accumulation outdoor. We build a large-scale cultivation technology. Two separation strategies were applied. Finally, a novel vegetable oil extraction technology of β-carotene was applied. Main conclusions were listed as the follows:

(1) Under indoor cultivation conditions：6000Lux, 30℃，7-8 cultivation time，using $NaNO_3$、KNO_3 as nitrogen source，NaCl to increase salty, the dry weight of *Dunaliella* can reach 0.13 g/d, the optimal conditions for the *Dunaliella* cells growth were under the nitrogen concentration of 5.0mM, salinity of 1.5M, and the orange light. The optimum conditions（22000Lux, 35℃） indoor for β-carotene accumulation were under nitrogen concentration of 0.5mM, salinity of 2.5M. β-carotene content was up to 3.64%, after 8 days of cultivation β-carotene yield was 3.035mg / (L·d).

(2) In outdoor cultivation（from 2015.05.29-2015.06.15，average illumination of 13000μw/cm^2）, using NaNO3、KNO3 as nitrogen source ， NaCl to increase salty，the best conditions for the β- carotene accumulation were 0.6mM nitrogen, 2.5M salinity. Accumulation of β- carotene content reached 3.14% and a yield of 2.47 mg/(L·d).

(3) Cells in normal cultivation method grew faster than the two-step cultivation method and the number of cells was approximately 21.05% higher, which suggested the extreme environment will inhibit the growth of *Dunaliella* cells. The accumulation of β- carotene was 33.2% higher than normal cultivation under the two-step method.

(4) Sand enhanced electric flocculation technology and used *spirulina* culture are

two effectively low harvesting technologies. When the electric current was 0.5A, speed of 150 rpm and sand dose was 250mg/L, the best recovery reached 97.16% and energy consumption was 0.380 kWh/kg, which was far below centrifugal separation (1.67 kWh/kg) applied in the industrial. Furthermore, the flocculated supernatant can be reused after supplement of nitrogen. The sand enhanced electric flocculation is a great way to save water and reduce the cost of cultivation. When the ratio of used *Spirulina* culture and *Dunaliella* broth is 1: 4, the recovery effect can reach 75.26%. Furthermore, we mixed the used *Spirulina* culture medium and fresh *Spirulina* culture medium, when the ratio of the mixture liquid and *Dunaliella* broth was 1:5, settling time was 3h, the optimum recovery was 74.77%. Using the supernatant of flocculated liquid to culture *Dunaliella* with the ratio of 2:5, the biomass of algal cells was 75% higher than normal cultivation.

（5）Microwave-assisted vegetable oil technology is a safe and effective method to extract β- carotene , when the reaction conditions is 50% power, 6min, 48℃, solid-liquid ratio of 1: 6, the highest recovery rate can reach 83.60 %, which is 23.27% higher than the direct vegetable oil extraction; the maximum recovery of 67.82% can be reached using vegetable oil direct extraction with the reaction conditions in 50℃, 40min, solid to liquid ratio of 1: 5.

Key words：*Dunaliella*; Large-scare cultivation; Harvesting; β-carotene;

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